OIPE SC177 SEP 17 2004 PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Bruno FLORIN et al.	Confirmation No.: 5455
Patent No.: 6,783,984 B2	Application No.: 09/826,393
Patent Date: August 31, 2004	Filing Date: April 3, 2001
For: PROCESS FOR THE CRYO-PRESERVATION OF PLANTS	Attorney Docket No.: 88265-4014

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. § 1.322

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Certificate
SEP 2 1 2004
of Correction

Sir:

Patentees hereby respectfully request the issuance of a Certificate of Correction in connection with the above-identified patent. The correction is listed on the attached Form PTO-1050, submitted in duplicate. The correction requested is as follows:

At column 14, line 36 (claim 7, line 3), after "between –20°C. and –40°C. before step of", delete "crupfreezing" and insert -- cryofreezing --. Support for this correction can be found in the Examiner's Amendment, attachment to Notice of Allowability, mailed May 26, 2004.

The requested correction is for an error that appears to have been made by the Office. Therefore, no fee is believed to be due for this request. Should any fees be required, however, please charge such fees to Winston & Strawn LLP Deposit Account No. 50-1814. Please issue a Certificate of Correction in due course.

Respectfully submitted,

9/17/04
Date

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.: 6,783,984 B2
DATED: August 31, 2004
INVENTORS: Florin et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 14:
Line 34, after "between –20°C. and –40°C. before step of", delete "crupfreezing" and insert -- cryofreezing --.

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TABLE 7

Cryo-preservation of carrot hypocotyl using a desiccation method

Starting conditions	Number of explants	After drying under 43% and freezing in LN: Rates of surviving explants (%)	After drying under 43% and freezing in LN: Rates of embryogenic explants (%)
Without culture on induction medium	30	23	13
After 3 week culture on induction medium	30	66	33

It is shown that the initial culture phase on the induction medium before freezing improves the success of the cryo-preservation of carrot primary explant.

From the above examples it could be clearly seen that cryo-preservation of primary explants capable to regenerate plants was successfully performed with cocoa, coffee and carrot species, a recalcitrant, semi-recalcitrant and orthodox species respectively. The primary explants used are pieces of flower bud, pieces of leaves and hypocotyl segments, respectively, according to the organ already known as capable to regenerate. Induction of freezing tolerance using a hardening-off treatment in presence of high sucrose concentrations has exemplarily been used. Various freezing methods can be used, i.e., the already described method with some simplifications as a pre-freezing step at preferably about −25° C. or a partial dehydration before freezing for species known to be desiccation sensitive (cacao, coffee) and a desiccation before freezing for carrot which is considered as desiccation tolerant species.

According to the present invention the time period to introduce an accession in a cryo-preserved gene bank could enormously be reduced while preserving the capability to regenerate into a plants after cryo-preservation. The process described allows to envisage long-term storage of genetic resources for large collection of different species and specially the tropical ones without the drawbacks inherent to the establishment and maintenance of in vitro culture.

What is claimed is:

1. A process for the cryo-preservation of a primary regeneration tissue derived from cocoa plant or a coffee plant comprising the following steps:

cultivating a plant tissue derived from cocoa plant or a coffee plant on an induction medium for a time sufficient to induce a primary regeneration tissue comprising embryogenic cells;

culturing the primary regenerating tissue on a multiplication medium for a time sufficient to maintain a stable proliferation of the primary regeneration tissue;

treating the primary regeneration tissue in a two step process that comprises sequential incubation in first and second sucrose media, wherein the second medium contains a greater amount of sucrose than the first medium;

prefreezing the primary regenerating tissue to a temperature between −20° C. and −40° C.; and

cryofreezing the primary regeneration tissue.

2. The process of claim 1, wherein the treating step further comprises a dehydration step and wherein the dehydration step involves placing the primary regeneration tissue in an air current of a laminar flow cabinet, in a stream of compressed air, or in an airtight container together with silica gel or various over-saturated salt solutions to control the relative humidity.

3. The process of claim 1, wherein the plant tissue utilized is derived from *Coffea canephora* or *Coffea arabica*.

4. The process of claim 1, wherein the plant tissue utilized is derived from *Theobroma cacao*.

5. A process for the cryo-preservation of a primary regeneration tissue derived from a cocoa plant or a coffee plant comprising the steps of:

incubating a plant tissue derived from a cocoa plant or a coffee plant in an induction medium for a time sufficient to induce a primary regeneration tissue comprising embryogenic cells;

pretreating the primary regeneration tissue by culturing the primary regeneration tissue on successive culture media with an increasing concentration of sucrose; and

cryofreezing the primary regeneration tissue.

6. The process of claim 5, further comprising the step of culturing the primary regeneration tissue on a multiplication medium for a time sufficient to maintain a stable proliferation of primary regeneration tissue before step of pretreating.

7. The process of claim 5, further comprising the step of prefreezing the primary regeneration tissue to a temperature between −20° C. and −40° C. before step of crupfreezing cryofreezing

8. The process of claim 5, wherein the step of pretreating the primary regeneration tissue comprises first incubating the primary regeneration tissue in a medium containing 0.4 M sucrose followed by incubating the primary regeneration tissue in a medium containing 1 M sucrose.

9. The process of claim 5, wherein the step of pretreating the primary regeneration tissue comprises culturing the primary regeneration tissue in medium containing 0.25 M sucrose, followed by culturing on a medium containing 0.5 M sucrose, followed by culturing on a medium containing 0.75 M sucrose, which is followed by culturing the primary regeneration tissue on a medium containing 1.0 M sucrose.

10. The process of claim 1, wherein the step of treating comprises first incubating the primary regeneration tissue in a medium containing 0.4 M sucrose followed by the incubation of the primary regeneration tissue in a medium containing 1 M sucrose.

11. The process of claim 1, wherein the temperature of the prefreezing step is minus 25° C.

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